

FIGURE 1. Sowing orchid seed with a syringe.

# MATERIALS AND METHODS

An experiment with starch as the gelling agent was carried out. Media constituents were coconut water (100 ml.l<sup>-1</sup>), 2g l<sup>-1</sup> of commercial fertilizer with 6:9:6 N:P:K concentrations, 20g 1<sup>-1</sup> of sucrose, 100g 1<sup>-1</sup> of banana pulp, and four cherry tomatoes. Two kinds of starch were tested, maize and cassava, both inexpensive and easy to find. The starch concentrations were 70, 90, and 110g l<sup>-1</sup>. Seeds were disinfected, in a syringe, with calcium hypochlorite at 0.5% concentration, washed twice with sterile water and sowed (FIGURE 1). The caps used to cover the flasks were either metal or polypropylene (Fig-URE 2). After two months, seedlings were replanted (FIGURE 3) in the same media with the aid of a forceps disinfected with 1% calcium hypochlorite.

## RESULTS

Media made with cassava starch did not reach the solid gel state. It was viscous even with  $110g\ l^{-1}$  and did not allow sowing. Maize starch media remained firm, and seedlings planted in it grew well. Despite not being equilibrated in the composition (Table 1), the maize medium is suitable for sowing and growing orchids from seed, especially *Cattleya* and their relatives and *Oncidium*.

In storing the flasks, both types of caps, the

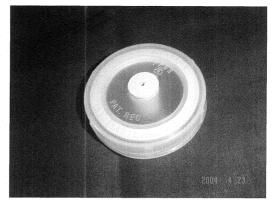


FIGURE 2. Polypropylene cap for media flask.

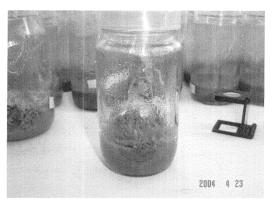


FIGURE 3. Orchid protocorms in starch medium, seedlings well developed and ready for replanting in same medium.

metal and the polypropylene, showed good results. After successive sterilization processes, however, the metal caps became oxidized and might release iron oxide to the media. For some orchid species, iron oxide can be toxic.

The results obtained were better when just a few seeds, ca. 40–50 per flask, were sown. Sowing with a syringe facilitated the process.

# CONCLUSION

If the purpose of sowing orchid seed is non-commercial, a media using starch as the gelling agent can be used with good results. The pH may be adjusted, as needed, with ammonia hydroxide or hydrochloric acid. This type of methodology promises to be of special interest to private growers, who are interested in raising their own orchids.

# FLORISTICS AND CONSERVATION OF ORCHIDACEAE AT RIO DAS PEDRAS RESERVE

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ABSTRACT. This work consists of surveying the floristics and conserving the Orchidaceae of the Rio das Pedras Reserve in the Mangaratiba municipal district of Rio de Janeiro. This area, located between the coordinates 22°59′S and 44°05′W, represents a valuable remnant of the Atlantic Pluvial Forest. Two subfamilies, 43 genera, and 88 species of orchids have been identified in the Reserve. The orchid family is the most representative one among the monocotyledons, representing 48% of the total species in this group. The most representative genera are *Pleurothallis* (13 spp.) and *Epidendrum* (9 spp.). A live collection of species from the Reserve, maintained by the Research Institute of the Botanical Garden of Rio De Janeiro, duplicates the taxa needed for listing species in the area. To date, 24 orchid species have been analyzed regarding their conservation status and placed in the following categories: Endangered (EN), Vulnerable (VU), Low Risk (LR), Rare (RARE), and Data Deficient (DD). In contrast to other surveys of the orchid family in the State of Rio De Janeiro, the Reserve is the smallest area but the richest in orchid species and thus an important forest remainder in the State.

Key words: Orchidaceae, floristic, conservation, Atlantic Forest, Brazil

#### Introduction

The Orchidaceae is among the largest of the plant families with ca. 20,000 natural species found in all parts of the world, especially in the tropics and subtropics (Barros 1990). Most of these plants are epiphytes, but they may also be rupicolous, terricolous, and saxicolous, as well as saprophytes. In Brazil, ca. 191 orchid genera have been identified (Pabst & Dungs 1975) along with 2400 species (Barros 1996).

Orchids have a close relationship with their pollinators, most of which are insects or birds attracted by the shape, color, and odor of the flowers. Many orchid species produce nectar and oils as rewards, but false rewards also insure pollination. Orchids have a symbiotic relationship with mycorrhizae, and nutrient uptake by these fungi is essential for germination of tiny orchid seeds, which lack an endosperm. As with the mycorrhizae, orchids are adapted to specific conditions; and this explains, in part, why some species have restricted ecological niches (IUCN 1996).

Many orchid species are endangered, because

of commercial over-collecting but also natural habitat destruction. Species diversity in this family is, therefore, an indicator of the state of conservation and/or regeneration of a certain habitat, especially in the Atlantic Forest, which is rich in epiphytes (Pinheiro 1999).

Conserving natural habitats will protect many species, including orchids; but some species require more specific measures (IUCN 1996). The conservation of plant species may depend on both in-situ and ex-situ strategies. In-situ conservation takes place in conservation units, while ex-situ conservation depends on seed banks, botanical garden collections, nurseries with testimonial seedlings, cryopreservation, and in-vitro collections. These methods complement one another, because not all wild species and their populations can be maintained in natural reserves or environmental protection areas, which often have been reduced to small areas by urban sprawl and expanding agricultural frontiers (Cavalcanti & Walter 1998).

The orchid collection maintained by the Research Institute of the Rio de Janeiro Botanical Garden aims to scientifically conserve and propagate genetic material from the Brazilian flora, thus contributing to the ex-situ conservation of

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endangered species. A data bank has been developed with information on this collection (Costa et al. 2002).

Current research objectives set by the authors include listing taxa and analyzing floristic diversity of the Orchidaceae from the Rio das Pedras Reserve (RPR) in Mangaratiba; promoting creation of a live collection (ex-situ conservation) of orchid species from the RPR at the Research Institute of the Rio de Janeiro Botanical Garden; and comparing floristic inventories of this family in areas of the Atlantic Forest in Rio de Janeiro State.

## MATERIAL AND METHODS

As a remnant of the Atlantic Rain Forest, the Rio das Pedras Reserve (RPR) lies within the city limits of Mangaratiba Municipality, Rio de Janeiro State, near Sepetiba Bay. Access to the area is from the BR-101 highway (Rio-Santos) at km 45. Geographic coordinates are 22°59'S and 44°05'W, with 20-1050 m elevation in an area of 1305 ha. The Reserve is designated as a Private Natural Heritage Reserve (RPPN in Portuguese) by the classification system of IBAMA (Brazilian Institute of the Environment and Renewable Natural Resources). The area lies within the Rio Grande watershed, and this river runs full course through a deep valley, descending sharply from source to mouth over steep terrain with a 37° slope (Vidal 1995).

The region has a warm climate with a mean annual temperature of 22°C, rainfall of 1200–2500 mm, and 11–12 months with 800 mm of hydric excess. Ten main trails cut through the reserve, which is considerably influenced by human activities, especially from ca. 20–420 m elevation, where bananas once were planted. Above 450 m, the forest becomes more dense and humid, with large trees forming a canopy ca. 20–30 m tall (Quinet 2002).

## **Floristics**

As part of our research, we revised the list of Orchidaceae species compiled by botanists from the Angiosperm Laboratory, Santa Ursula University (USU), who have been collecting in the area since 1996 as part of the Rio das Pedras Reserve Floristic Project.

From February 2003 to February 2004, we made monthly 3-day trips to the study area. We made botanical collections along the trails and in the surroundings, giving preference to flowering and fruiting material. We recorded data on morphology, population numbers, phenology, life forms, substrate type, and altitude. Flowers were fixed in 70% alcohol with 10% glycerin

for later analysis (Pinheiro 1999). Specimens were pressed and dried according to standard procedures (Fidalgo & Bononi 1984) and deposited in the Santa Ursula University Herbarium (RUSU). Whenever possible, duplicates were sent to the Rio de Janeiro Federal University Herbarium (RFA) (abbreviations follow Holmgren et al. 1990).

Data on major plant groups in the study area are presented. Taxonomic studies were based on the literature as well as consultations with specialists and with the collections of the Research Institute, Rio de Janeiro Botanical Garden. The classification system follows Dressler (1993).

Species lists containing references to the conservation status of Brazilian orchids were analyzed using IUCN criteria (1994): Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Low Risk (LR), Rare (RARE), and Deficient Data (DD).

#### **Ex-Situ Conservation**

Vegetative specimens are deposited in the Live-Plant Collection of the Rio de Janeiro Botanical Garden Research Institute (JBRJ). For this purpose, we collect specimens that have fallen to the ground and those that will potentially be new additions to the species list of the area. Specimens of plants that flower in cultivation are preserved, as described above.

## **Comparative Floristics**

We analyzed a number of orchid surveys carried out in Atlantic Forest remnants in the state of Rio de Janeiro. Surveys were selected to facilitate comparison with the Rio das Pedras Reserve regarding size of the area, collection effort, number of species, and methodology.

# RESULTS AND DISCUSSION

The orchid species list compiled by the team of the Rio das Pedras Reserve (RPR) Floristic Project originally had 29 species and 22 genera of Orchidaceae. After one year of collecting in the area, the list grew to 88 species in 43 genera (Table 1). Of this total, 59 species were collected solely by the senior author, 17 by the project team, and 12 by both (FIGURE 1).

Orchidaceae make up 48% of the monocots found in the RRP, followed by Araceae (10%), Marantaceae (9%), Commelinaceae (8%), Bromeliaceae (8%), and other families (17%).

The Orchidaceae consists of five subfamilies (Dressler 1993), three of which have representatives in Brazil. The orchid species at RPR belong to two subfamilies—Epidendroideae and

TABLE 1. Orchid checklist of Rio das Pedras Reserve, Mangaratiba, RJ, Brazil.

Orchid species	Orchid species		
Beadlea bicolor (ker Gawl.) Garay	Maxillaria marginata (Lindl.) Fenzl.		
Beadlea elata (Sw.) Small	Maxillaria parviflora (Poepp. & Endl.) Garay		
Beadlea elegans (Hoehne) Garay	Maxillaria picta Hook.		
Beadlea itatiaiensis (Krzl.) Garay	Maxillaria rufescens Lindl.		
Beadlea aff. longibracteata (Barb. Rodr.) Garay	Maxillaria sp. 1		
Bifrenaria harrisoniae (Hook) Rchb.f.	Mesadenella cuspidata (Lindl.) Garay		
Bifrenaria inodora Lindl.	Octomeria albopurpurea Barb. Rodr.		
Bulbophyllum micranthum Barb. Rodr.	Octomeria juncifolia Barb. Rodr.		
Campylocentrum sellowii (Reichb.f.) Rolfe	Octomeria oxychella Barb. Rodr.		
Cattleya forbesii Lindl.	Octomeria tricolor Rchb.f.		
Cirrhaea saccata Lindl.	Octomeria sp.		
Cochleanthes sp.	Oeceoclades maculata (Lindl.) Lindl.		
Constantia sp.	Oncidium cornigerum Lindl.		
Cyrtopodium polyphyllum (Vell.) Pabst ex F.Barros	Oncidium fimbriatum Lindl.		
Dichaea cogniauxiana Schltr.	Oncidium flexuosum Sims.		
Dichaea pendula (Aubl.) Cogn.	Oncidium longipes Lindl.		
Dipteranthus pellucidus (Rchb.) Cogn.	Pelexia novofriburgensis (Rchb.f.) Garay		
Elleanthus sp.	Pleurothallis aphtosa Lindl.		
Eltroplectris janeirensis (Porto & Brade) Pabst	Pleurothallis bidentulla Barb. Rodr.		
Eltroplectris triloba (Lindl.) Pabst	Pleurothallis compressiflora Barb. Rodr.		
Encyclia patens Hook.	Pleurothallis granulosa (Barb. Rodr.) Cogn.		
Epidendrum armeniacum Lindl.	Pleurothallis grobyi Bateman ex. Lindl.		
Epidendrum campaccii Hágsater & L. Sánchez	Pleurothallis hypinicola Lindl.		
Epidendrum filicaule Lindl.	Pleurothallis lineolata (Barb. Rodr.) Cogn.		
Epidendrum geniculatum Herb. Ham. Ex Hook.f.	Pleurothallis rubens Lindl.		
Epidendrum ramosum Jacq.	Pleurothallis saundersiana Rchb.f.		
Épidendrum rigidum Jacq.	Pleurothallis aff. trifida Lindl.		
Epidendrum secundum Jacq.	Pleurothallis sp. 1		
Epidendrum vesicatum Lindl.	Pleurothallis sp. 2		
Epidendrum sp.	Pleurothallis sp. 3		
Erythrodes arietina Ames	Polystachya concreta (Jacq.) Garay		
Eurystyles cogniauxii (Krlz.) Pabst	Prescottia plantaginea Lindl.		
Eurystyles cotyledon Wawra	Promenea ovatiloba Cogn.		
Galeandra beyrichii Reichb.f.	Promenea paulensis Schltr.		
Gomesa crispa Klotzch ex Reichb.f.	Prosthechea pigmaea (Hook) W.E. Higgins		
Gomesa planifolia Klotzch ex Reichb.f.	Prosthechea vespa (Vell.) W.E. Higgins		
Gongora bufonia Lindl.	Sacoila lanceolata (Aubl.) Garay		
Hapalorchis lineata Schltr.	Sarcoglottis fasciculata (Vell.) Schltr.		
Isochilus linearis (Jacq.) R. Br.	Stanhopea guttulata Lindl.		
Laelia sp.	Stelis megantha Barb. Rodr.		
Lankesterella ceracifolia (Barb. Rodr.) Mansf.	Stelis aff. modesta Barb. Rodr.		
Leptotes bicolor Lindl.	Tetragamestus modestus Rchb.f.		
Malaxis excavata Kuntze	Wullschlaegelia aphylla Reichb.f.		
Maxillaria brasiliensis Brieger & IIIg.	Xylobium variegatum (Ruiz & Pav.) Garay & Duns		

Spiranthoideae—which are mostly epiphytes or terrestrial species, respectively. Of the 43 genera, the most species-rich are *Pleurothallis* (13 spp.), *Epidendrum* (9 spp.), *Maxillaria* (6 spp.), *Beadlea* (5 spp.), *Octomeria* (5 spp.), *Oncidium* (4 spp.), *Bifrenaria* (2 spp.), *Dichaea* (2 spp.), *Eltroplectris* (2 spp.), *Eurystyles* (2 spp.), *Gomesa* (2 spp.), *Promenea* (2 spp.), *Prosthechea* (2 spp.), and *Stelis* (2 spp.); and the other genera have one species each (FIGURE 2). According to Dressler (1993), *Pleurothallis* and *Epidendrum* are strictly neotropical genera with c. 1120 and 800 species respectively. These are the two larg-

est genera in the orchid family led by Pleuro-thallis.

Regarding life form, 61 of the 88 species analyzed are strictly epiphytes, 20 are strictly terrestrial, and 7 are epiphytes and terrestrial (FIGURE 3). The terrestrial species are classified as terricolous (12 spp.), rupicolous (4 spp.), saxicolous (3 spp.), and saprophytes (1 sp.) (FIGURE 4).

Of the 61 strictly epiphytic species, 57 belong to the subfamily Epidendroideae. Although most of the genera in this subfamily have only epiphytic species, some genera, such as *Malaxis* 

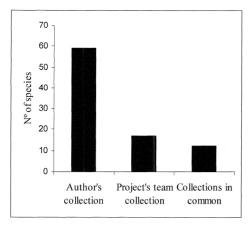


FIGURE 1. Collection of orchid species in Rio das Pedras Reserve, Mangaratiba, RJ, 2003–2004.

and *Oeceoclades*, include terrestrial species (Fraga 2000). Advantages of the epiphytic habit include better light conditions and a relatively competition-free substrate (Waechter 1986 apud Fraga 2000). According to Madison (1977 apud Dressler 1981), epiphytes are more readily exposed to pollinators and have better seed dispersal. Epiphytism, as a speciation mechanism that facilitates finding better habitats, contributed to the expansion of the Orchidaceae, which has given rise to a marked increase in diversity in tropical forests (Benzing 1981, 1987; Gentry & Dodson 1987 apud Fraga 2000). According to Dressler (1981), epiphytic orchids are restricted to tropical and subtropical habitats.

Of the 20 terrestrial species, 12 belong to the Spiranthoideae, which represents the life form most commonly found in this subfamily.

All seven species that have both terrestrial

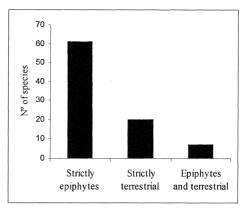


FIGURE 3. Number of species of Orchidaceae distributed according to their life form in Rio das Pedras Reserve, Mangaratiba, RJ.

and epiphytic life forms are found on the banks of the Rio Grande. Two of these species are found in other habitats (forest interior or rocky outcrops), where they always occur as one life form. For instance, *Epidendrum secundum* Jacq. is a terrestrial plant growing on rocky outcrops, and *Isochilus linearis* (Jacq.) R. Br. is an epiphyte in the forest. According to Dressler (1981), under favorable conditions, many epiphytic orchids are found growing as terrestrial plants, and terrestrial plants as epiphytes. Fraga (2000) mentions 12 species that are both epiphytic and terrestrial or cited in the literature as epiphytes but collected on the sandy coastal plains of Espírito Santo as terrestrial plants.

# **Conservation Status**

Endangered plant species have been listed for several states and municipalities in Brazil. Ref-

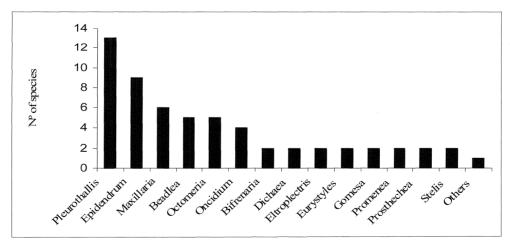


FIGURE 2. Richest genera of Orchidaceae in Rio das Pedras Reserve, Mangaratiba, RJ.

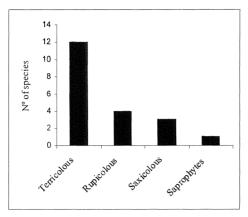


FIGURE 4. Number of terrestrial species of Orchidaceae, categorized as terricolous, rupicolous, saxicolous, and saprophytes in Rio das Pedras Reserve, Mangaratiba, RJ.

erences were found for Rio Grande do Sul (RS), Paraná (PR), Rio de Janeiro Municipality (RJ), and for the sandy coastal plains of Espírito Santo (ES). References to conservation status were found for 24 species in listings by the RRP. Nine species fall into the category Endangered (EN), six are Vulnerable (VU), five are Low Risk (LR), one is Rare (RARE), and two have deficient data (DD). *Bifrenaria harrisoniae* (Hook) Rchb.f. was classified as Endangered in RS and RJ Municipality, while *Cyrtopodium polyphyllum* (Vell.) Pabst ex F. Barros is endangered in RS and Low Risk on the sandy coastal plains of ES. Sixteen of the 24 species are being cultivated at JBRJ, where, in the future, they can be utilized for propagation actions, re-introduction, and education (TABLE 2).

## Live Collections and Ex-Situ Conservation

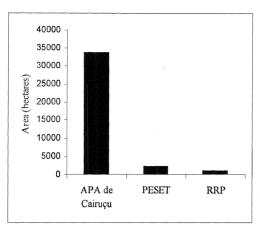
The live orchid collection of the RPR, which was set up at the JBRJ Orchid House, today contains 124 specimens belonging to 38 genera and 52 species. Some 72 of these plants have not yet flowered and have only been identified to the genus level.

Of the 88 species that make up the list of orchids at RPR, 45 are under cultivation (51% of the total number of species). Thirteen species (15% of the total) have not yet flowered and have only been identified to the genus level. Be-

TABLE 2. Conservation status of 24 species frm the checklist of Rio das Pedras Reserve, Mangaratiba, RJ. Locations: RS (Rio Grande do Sul State), PR (Paraná State) Rio de Janeiro (Rio de Janeiro City), and ES (Sand Coastal Plains from Espirito Santo State). Status: DD (deficient data), EN (endangered), LR (low risk), RARE (rare), and VU (vulnerable). Sources: Banco de Dados Tropical (BDT 2004), Secretaria Municipal de Meio Ambiente (SMAC 2000), and Fraga (2000).

Sancias	Status per locality				
	DC	PR	Rio de Janeiro	EC	
Species	RS	PR	Janeiro	ES	JBRJ Greenhouse
Beadlea elegans				VU	
Bifrenaria harrisoniae	EN		EN		
Bifrenaria inodora	EN				X
Cattleya forbesii			EN		X
Cirrhaea saccata	EN				X
Cyrtopodium polyphyllum	EN			LR	X
Eltroplectris triloba				LR	
Epidendrum rigidum				VU	
Epidendrum secundum				DD	$\mathbf{X}$
Erythrodes arietina	EN				X
Gongora bufonia		RARE			X
Hapalorchis lineata		EN			
Leptotes bicolor		EN			X
Mesadenella cuspidata				VU	X
Oeceoclades maculata				LR	
Pleurothallis auriculata				EN	X
Pleurothallis grobyi				VU	X
Pleurothallis saundersiana				VU	X
Polystachya concreta				LR	X
Prescottia plantaginea				LR	X
Prosthechea pygmaea				VU	
Sacoila lanceolata				DD	X
Sarcoglottis fasciculata				LR	X
Wullschlaegelia aphyla		EN		DIC	21

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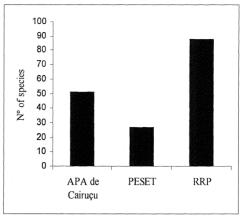


FIGURE 5. Comparisons of Rio das Pedras Reserve (RRP), Cairuçu Environmental Protection Area (APA), and Serra da Tiririca State Park (PESET): A. Area. B. Species richness.

sides adding a significant number of taxa to the list, the live collection has allowed us to observe the development and morphological aspects of vegetative and reproductive organs of the species, which will contribute to future studies.

Live collections are essential for conservation, especially regarding propagation and reintroduction of orchid species. These plants produce thousands of seeds, and there are well-known techniques for their symbiotic and asymbiotic germination. Such activities must be closely followed by studies of geographic distribution, habitat specificity, and population size of each species (IUCN 1996). When this type of conservation is linked to nature education, it plays a major role in the protection of orchid species (Rivero 2001). Educational programs related to orchid collections are needed, especially in the tropics, because otherwise conservation efforts will not bear fruit (Davis et al. 1986).

#### **Comparing Other Floristic Lists**

A number of floristic surveys in the state of Rio de Janeiro deal with Orchidaceae in conservation units. These include Itatiaia National Park in Itatiaia (Brade 1951), Macaé de Cima Ecological Reserve in Nova Friburgo (Miller & Warren 1994), Serra da Tiririca State Park in Niterói and Marica (Pinheiro 1999), Cairuçu Environmental Protection Area in Parati and the JBRJ Atlantic Forest Program team (Marques 1997), Massambaba (Fagnani & Siqueira 1998), and Ilha de Cabo Frio in Arraial do Cabo (Mello 2002).

We selected the lists prepared by Pinheiro

(1999) and Marques (1997) for comparison with that by the RPR, considering the parameters used in each study. All three areas are near the ocean and have rocky outcrops, streams, and similar altitudinal variation. Regarding area and number of species, the Cairuçu Environmental Protection Area has 33,800 ha and 51 species (Marques 1997), Serra da Tiririca State Park (PESET) has 2400 ha and 27 species (Pinheiro 1999), and RPR has 1305 ha and 88 species (FIGURE 5).

Comparing area size with number of species per unit shows that RPR has the smallest area and the highest number of species. This may be the result of methodology that has combined fieldwork with cultivation, increasing the number of taxons sampled by ca. 50%. Other factors, such as collection effort and state of forest preservation at the RPR and surrounding area, contributed to the high species richness recorded for this unit. The Orchidaceae, however, are still in need of a more detailed assessment, considering that the highest point in the Reserve at 1050 m has yet to be explored for orchids.

Although the Cairuçu Environmental Protection Area has a total area of 33,800 ha, ca. 6750 ha are given over to farming, and 8110 ha are secondary forest (Marques 1997). These factors directly influence the state of preservation in the area, yet ca. 17,240 ha of dense rain forest remain (Marques 1997). The number of species found in the area (51) is rather low when compared to RPR (88), but collection efforts were not focused on Orchidaceae, and no effort was made to set up a live collection for the species found in the vegetative state.

PESET is also an area that suffers from pressures such as deforestation, poaching, fire, and real estate development. Furthermore, most of the area is covered by secondary forest in various stages of regeneration, and the surrounding area is being deforested, which has led to regional climate change (Pinheiro 1999). Although collection efforts were geared toward orchids, and a live collection was made, few species were recorded (26). This low number may indicate that the family was not well sampled or that the species have in fact suffered from existing pressures and have declined in the area. Many orchids adapt to specific microclimates and are sensitive to environmental change; these may disappear in certain spots where the populations have declined (IUCN 1996).

Both ex-situ and in-situ conservation need to be considered when studies are carried out in conservation units, as the basis for various lines of research, including nature education. Improving our knowledge of reproductive biology and ecology of plant populations, especially those threatened with extinction, is essential to provide the basis for reintroduction of these plants in conservation units.

Our research suggests that collection efforts focusing on a single family produce more consistent results, which indicates that training specialists will guarantee better representation of certain groups of plants, especially families with high species numbers.

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